

Comparative cranial morphology of douc langurs (*Pygathrix cinerea*, *P. nemaeus*, *P. nigripes*)

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Key words: *Pygathrix*, cranial morphology, comparison

Summary

The study describes the cranial morphology of the three species of douc langurs, *Pygathrix cinerea*, *P. nemaeus* and *P. nigripes*. The variability of some features could be analysed on 24 skulls of *P. cinerea*, 18 skulls of *P. nemaeus*, and 4 skulls of *P. nigripes*.

28 linear measurements were taken on all skulls for statistical analyses. As the skulls clearly change with age, correlations between each measurement and age have to be eliminated from statistical analyses. Therefore, age-independent residuals were calculated from all linear measurements in linear regressions against the age class.

Morphologically the skulls of the three species of *Pygathrix* can not be clearly separated from each other by naked eye or simple measurements. Student's t-tests revealed only few statistically significant differences between the species but none in the age-independent residuals between *P. cinerea* and *P. nemaeus*. Discriminant analyses of the age-independent residuals indicated the possibility to separate the three species of *Pygathrix*.

So sánh đặc điểm giải phẫu hình thái sọ ở các loài vọc chà vá (*Pygathrix cinerea*, *P. nemaeus*, *P. nigripes*)

Tóm tắt

Nghiên cứu này mô tả các đặc điểm giải phẫu hình thái sọ của ba loài chà vá tại Việt Nam gồm *Pygathrix cinerea*, *P. nemaeus* và *P. nigripes*. Sự biến thiên của các đặc điểm giải phẫu sọ được phân tích trên 24 mẫu sọ của loài *P. cinerea*, *P. nemaeus* (18 mẫu) và loài *P. nigripes* (4 mẫu). 28 thông số đã được đo và được phân tích thống kê. Khi xử lý số liệu thống kê, mối quan hệ giữa các số đo và độ tuổi của mẫu sọ phải được loại bỏ. Nhờ đó, các biệt số độc lập với độ tuổi được tính từ tất cả các phép đo trong thống kê hồi quy tuyến tính.

Hình thái của các mẫu sọ của ba loài thuộc giống *Pygathrix* không thể phân biệt rõ bằng mắt thường và các thông số đo đơn giản. Kiểm định thống kê T-test cho thấy chỉ có một vài sự sai khác một cách có ý nghĩa thống kê các số đo của mẫu sọ thuộc hai loài *P. cinerea* và *P. nemaeus*. Tuy nhiên, phân tích thống kê sự khác biệt của biệt số độc lập với độ tuổi cho thấy có thể phân loại 3 loài chà vá thuộc giống *Pygathrix* dựa trên đặc điểm hình thái sọ.

Introduction

Vietnam is considered a “Biodiversity Hotspot” (Mittermeier et al., 2004) and is characterized by a large number of endemic species (Stirling & Hurley, 2005; Sterling et al., 2006), including five of the world’s 25 most endangered primates (Mittermeier et al., 2009). One of them is the grey-shanked douc langur, *Pygathrix cinerea*, which has only recently been described, first as subspecies of the red-shanked douc langur, *P. nemaeus*, on differences in coloration (Nadler, 1997) and later was considered a distinct species on the basis of molecular genetic data (Roos & Nadler, 2001; Roos, 2004). Thus currently three species of *Pygathrix* are distinguished.

Pygathrix cinerea is listed as a “Critically Endangered” species, *P. nemaeus* and *P. nigripes* as “Endangered” (IUCN, 2009). All three species are relatively poorly known.

P. nemaeus, occurs in central to south Laos, north-central Vietnam and in a small area in north-east Cambodia (Rawson & Roos, 2008). *P. cinerea* occurs in five provinces in central Vietnam: Quang Nam, Gia Lai, Kon Tum, Quang Ngai and Binh Dinh (Ha Thang Long, 2007; Nadler et al., 2003; 2007), and probably in restricted areas in Laos and Cambodia close to the Vietnamese border (Duckworth et al., 1999; Rawson & Roos, 2008). *P. nigripes* occurs in east Cambodia, east of the Mekong, and in southern Vietnam (Smith, 2001; Nadler et al., 2003; Pollard et al., 2007). The range of *P. cinerea* overlaps in small areas with *P. nemaeus* in central Vietnam and probably also in South-east Laos and north-east Cambodia (Ha Thang Long, 2004; Nadler et al., 2007; Rawson & Roos, 2008). Contact areas between *P. cinerea* and *P. nigripes* – if exist - are not clarified yet (Fig. 1).



Fig.1. Distribution of the three *Pygathrix* species according to Duckworth et al. (1999); Ha Thang Long (2004); Nadler et al. (2007); Pollard et al. (2007); Rawson & Roos (2008).

So far detailed data on the skull morphology and changes in craniometry during ontogeny are lacking besides of the comparison of cranial dimensions of *P. nemaeus* with *Rhinopithecus* (Pan & Oxnard, 2001). A first craniometric comparison of the three *Pygathrix* species is made by Harding (2003). The result of the study shows significant differences in size between the three taxa, but this is based on a very limited number of specimens (*P. nemaeus* 7 male, 5 female; *P. nigripes* 8 male, 2 female; *P. cinerea* 2 male, 2 female) and individual variation.

In this paper we describe the cranial morphology of the three species of douc langurs and assess ontogenetic changes with age. In this respect also the sequence of tooth replacement will be briefly considered.

It is aimed to check if cranial features could reliably be used to distinguish between the species now established on the basis of fur coloration and molecular genetic data. The morphological description is supplemented with a multivariate statistical analysis of linear cranial measurements and both are used to test if a separation of the three species of *Pygathrix* might be possible.

Material and Methods

The study is based on 24 skulls of *P. cinerea*, 18 skulls of *P. nemaeus*, and 4 skulls of *P. nigripes* from Endangered Primate Rescue Center (EPRC), the Zoological Museum at National University Hanoi (ZMNU), and Museum für Tierkunde Dresden (MTD) (Table 1).

Table 1. Studied material of *Pygathrix* species at the Endangered Primate Rescue Center (EPRC), Zoological Museum National University Hanoi (ZMNU), and Museum für Tierkunde Dresden (MTD) with assigned age groups.(1 = juvenile; 2 = subadult; 3 = adult)

species	coll. No.	sex	age	age group
<i>Pygathrix cinerea</i>	EPRC 7-07	f		2
<i>Pygathrix cinerea</i>	EPRC 7-33	m	8 years	3
<i>Pygathrix cinerea</i>	EPRC 7-26	f		3
<i>Pygathrix cinerea</i>	EPRC 7-18	m	5 years	2
<i>Pygathrix cinerea</i>	EPRC 7-02	m		3
<i>Pygathrix cinerea</i>	EPRC 7-17	f		3
<i>Pygathrix cinerea</i>	EPRC 7-06	m	9 years	3
<i>Pygathrix cinerea</i>	EPRC 7-08	m	10 years	3
<i>Pygathrix cinerea</i>	EPRC 7-27	f		3
<i>Pygathrix cinerea</i>	EPRC 7-10	f		3
<i>Pygathrix cinerea</i>	EPRC 7-15	m		1
<i>Pygathrix cinerea</i>	EPRC 7-12	m	1 year	1
<i>Pygathrix cinerea</i>	EPRC 7-20	m	5 years	2
<i>Pygathrix cinerea</i>	EPRC 7-27	f		2
<i>Pygathrix cinerea</i>	ZMNU M870	m		3
<i>Pygathrix cinerea</i>	ZMNU M850	m		3
<i>Pygathrix cinerea</i>	ZMNU 877	m		3
<i>Pygathrix cinerea</i>	ZMNU M869	m		3
<i>Pygathrix cinerea</i>	ZMNU M867	m		3
<i>Pygathrix cinerea</i>	ZMNU M855	f		3
<i>Pygathrix cinerea</i>	ZMNU M732a	m		3
<i>Pygathrix cinerea</i>	ZMNU M849	f		3
<i>Pygathrix cinerea</i>	ZMNU M854	m		1
<i>Pygathrix cinerea</i>	ZMNU M853	m		1
<i>Pygathrix nemaeus</i>	MTD B19855	m		3
<i>Pygathrix nemaeus</i>	EPRC 6-40	f		3
<i>Pygathrix nemaeus</i>	EPRC 6-44	m		3
<i>Pygathrix nemaeus</i>	EPRC 6-17	f	10 years	3
<i>Pygathrix nemaeus</i>	EPRC 6-25	m	3,5 years	1
<i>Pygathrix nemaeus</i>	EPRC 6-22	m		1
<i>Pygathrix nemaeus</i>	EPRC 6-11	m	5 days	1
<i>Pygathrix nemaeus</i>	EPRC 6-43	m	8 months	1
<i>Pygathrix nemaeus</i>	EPRC 6-27	m	3 years	1
<i>Pygathrix nemaeus</i>	EPRC 6-18	f	3 years	1
<i>Pygathrix nemaeus</i>	EPRC 6-19	m	2 years	1
<i>Pygathrix nemaeus</i>	EPRC 6-X03	m		1
<i>Pygathrix nemaeus</i>	EPRC 6-20	f	1 year	1
<i>Pygathrix nemaeus</i>	EPRC 6-24	f	3,5 years	1
<i>Pygathrix nemaeus</i>	EPRC 6-08	m	5...6 years	2
<i>Pygathrix nemaeus</i>	MTD B19854	m		2
<i>Pygathrix nemaeus</i>	ZMNU M 101	m		2
<i>Pygathrix nemaeus</i>	ZMNU M 1038	?		2
<i>Pygathrix nigripes</i>	EPRC 13-04	f	2,5 years	1
<i>Pygathrix nigripes</i>	EPRC 13-06	f	2 years	1
<i>Pygathrix nigripes</i>	MTD B19857	f		3
<i>Pygathrix nigripes</i>	MTD B19856	f		2

The skulls of subadult and adult animals at the EPRC come from animals that were rescued but subsequently died though heavy injuries or fatal digestion disturbance. Skulls of newborn and very young animals are from animals born at the EPRC and did not survive. Skulls from other collections are mostly from hunted animals.

The ages of individuals which were born at the EPRC is exactly known. The age of confiscated subadult and adult animals are estimated in comparison to animals at the EPRC with known age.

28 linear measurements were taken on the skull and mandible, partially in accordance to Maryanto et al. (1997). They are explained in Table 2 and illustrated in Fig. 2. All measurements were taken with digital callipers to the nearest 0.01 mm.

The morphological description follows Whitehead et al. (2002).

Table. 2. Measurements taken on cranium and mandible (see also Fig. 2.)

cranium	
gsl	greatest skull length, measured parallel to the base of the skull, including incisor
ch	cranial height, measured as maximal height above auditory bullae and basioccipital
zw	zygomatic width, maximal width across zygomatic arches
io	interorbital constriction, smallest distance between orbits
pob	postorbital breadth, measured at the narrowest point of the cranial constriction in the temporal fossa
orbw	orbital width
maxb	width across maxillas, or maximal rostral breadth, breadth across the maxillas
measure	d linguallly, dorsal to M1 (about equal to palatal breadth plus teeth)
pmxb	width across premaxillae, breadth across praemaxillary bones measured frontally at alveoles of canines
nb	nasal breadth
nl	nasal length
dM3	distance between M3s
dC	distance between canines
mfw	width across mesopterygoid fossa
dcc	distance between carotid canals
linfm	distance posterior rim of incisor to anterior end of foramen magnum
lip	distance of posterior rim of incisor to posterior end of palatine
libasio	distance of posterior rim of incisor to anterior end of basoccipital
gcw	greatest cranial width at hindcranium
lvom	length of vomer visible in choana
mandible	
dl	dentary length, measured parallel to the long axis of the mandible
pcr	maximal height of mandible, measured in straight line from coronoid process to base of angular process
pcp	condylar height, measured from the top of condyle to the base of angular process
mcorb	maximal length of ascending mandibular ramus, measured parallel to long axis of mandible
mandh	mandible height, height of mandibular ramus from ventral rim to alveole level between m1 and m2
mandw	width of mandible, measured across the mandible below m2/m3
dcond	condylar distance, distance between the condyles of right and left dentary in straight line
lcm3	length of mandibular tooth row, measured occlusally
cbf	width across both canines, measured frontally

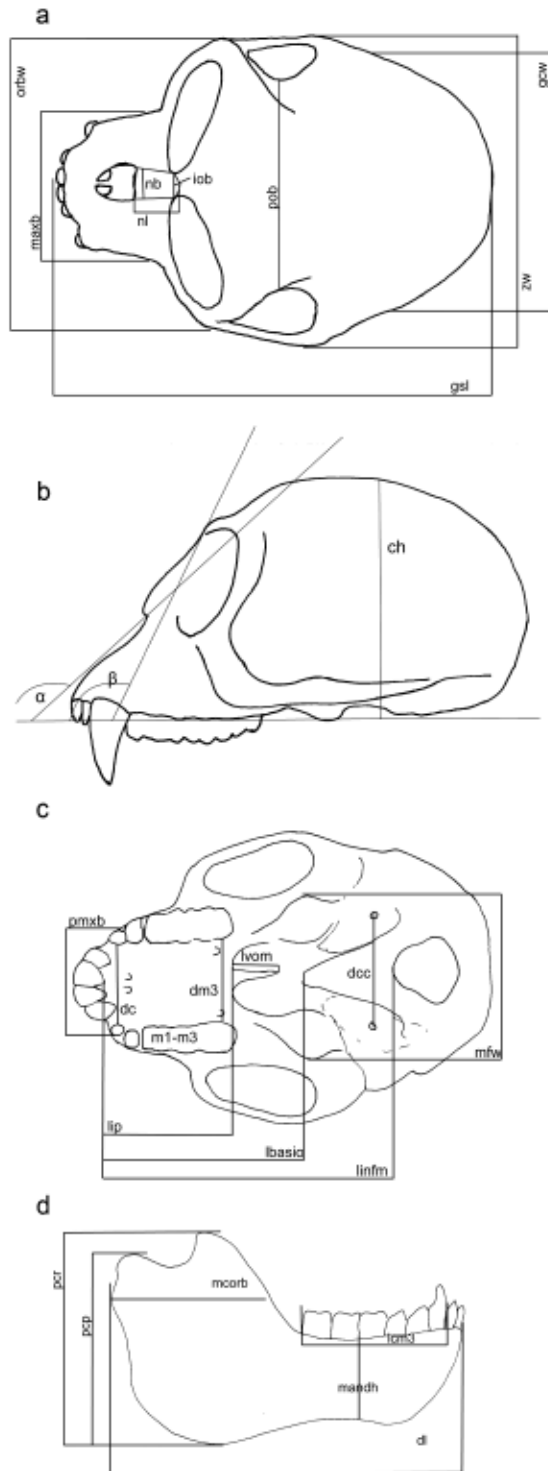


Fig.2. Schematic illustration of most of the measurements taken on the skull and mandible (Abbreviations see Table 2).

a. Skull in dorsal view; **b.** Skull in lateral view; **c.** Skull in ventral view (α : angle of snout and β : angle of orbital plane - not measured in each skull); **d.** Mandible in lateral view.

Aging and forming of age groups

Not many data on the life history of douc langurs are known to be used as a basis to form age groups suitable for statistical analysis. Tooth replacement, tooth wear and cementum apposition are often used to age mammals on the basis of cranial material. The most prominent example where teeth, and in particular tooth replacement and wear of the incisors, are used in age determination (e.g. Habermehl, 1961; Richardson et al., 1995; Muylle et al., 1997). Also for some economically important species age determinations on the basis of teeth have been given, e.g. for domestic cattle, *Capra hircus* or *Cervus elaphus* (Habermehl, 1961). In Japanese Macaques also cementum apposition was used for aging (Wada et al., 1978). The most thorough compendium on the ages of dental eruption in primates is given by Smith et al. (1994). An overview, based on Harvati (2000), Schultz (1935) and Smith et al. (1994) is provided by Swindler (2002).

The completion of dental development does not necessarily co-occur with sexual maturity and thus adulthood or full growth of the animal. For *Trachypithecus cristatus* males it has been stated that the dental development is concluded at the age of about 3.75 years (Harvati, 2000). In the slightly smaller males of *Macaca fascicularis* dental development is completed at the age of 5.5 years (Smith et al., 1994). Only few individuals of each species are of exactly known age, and only few of age <2 years which can be considered a very important time span for growth. For *P. cinerea* and *nemaeus* cranial growth reaches the adult level at about 50 months, about 4 years of age

Due to the difficulties of exact aging we established four age groups used in some of the statistical analysis: Table 1 gives the frequency of specimens in each age class for the species.

- 0 - newborns that died within a few days of birth (only 3 specimens),
- 1 - juveniles, young animals from about 8 months to 4 years of age,
- 2 - subadult about 4 to 5 years of age and
- 3 - adults from 6 years and more of age, definitely with a complete dentition.

Statistics

Reasonable statistical comparisons are only possible between *P. cinerea* and *P. nemaeus* as only four skulls of *P. nigripes* were available for analysis. They are included in the discriminant analysis, but the results concerning this species have to be treated with some caution.

All original data were checked for normal distribution using Q-Q Plots. As expected all measured parameters correlate strongly with age. Therefore, a method had to be found to eliminate the influence of age from the data. Here the same procedure as in Stefen & Rudolf (2007) is used. Linear regressions of all measured parameters against age – the four age groups – were performed, calculating the resulting unstandardized residuals of each variable against the independent age. These were saved as 'RES-variable' of the original measurement and used in further tests.

Two other ways to deal with the different ages of the specimens were used and finally all were compared. Analyses with the original data were done using specimens of age class 2 and 3 only. And the original data were log transformed and saved as 'Log variable' and these were used in different analyses.

Students t-tests (always at the 95 % significance level) were performed with the age-independent residuals a) to test for differences between females and males in each species and b) to test for differences between the species. In these tests *P. nigripes* was not used due to the low number of available specimens.

To see which variables are responsible for most of the variance in the overall sample a principal component analysis (PCA) was performed with the original data, the age-independent residuals and the log-transformed variables.

Discriminant analysis (DA) of the three species were also performed primarily with variables mainly influencing the first factor of the PCA and some different sets of the original data, the age-independent residuals or the log-transformed variables in order to see whether the species could be separated. The DAs were done using Wilk's lambda statistic, entry of all variables at once not stepwise, with equal prior probabilities of group membership, based on the pooled within-group covariance matrix. SPSS 16 was used for all statistical calculations.

Results

Description of juvenile skull of *Pygathrix*

Cranium

The description is mainly based on two skulls of *P. nemaeus* females (EPRC 6-18 and 6-20), but observations on other skulls are included.

In dorsal view the skulls are more or less oval to egg shaped being pointed at the interorbital constriction, with the orbital region forming a kind of base and a short snout attached (Fig. 3a). The frontoparietal suture is convex and in the sagittal plane the frontal extends over more than half of the skull length. The frontoparietal suture (*sutura coronalis*) might be neat and just curved or zigzagging irregularly especially in the middle of the skull. Distal and posterior to the orbital ridges, which are visible but not clearly separated from the rest of the frontals as in adult skulls, the frontal is well inflated, forming the highest point of the juvenile skull. The vault slightly curves ventrally to the posterior end of the skull. The parietooccipital suture (*sutura lambdoidea*) is just visible in the posterior end of the skull.

The postorbital constriction is shallow in these juvenile skulls and separates the orbital plane from the rest of the skull. The orbital width is smaller than the cranial width. The orbital plane is tilted in an angle β of 80° (see Fig. 2b) to the base of the skull toward posterior.

In frontal view the orbits dominate the impression of the skull and the cranium is well inflated. The nose opening is skewed oval, being more pointed ventrally, with the widest lateral extension more dorsal than the middle of its length and a nearly straight or levelled dorsal end at the nasals (Fig. 3b). Although the orbits appear well rounded, the orbital rims might be straightened in some skulls (e.g. MTD B19400) and the lateral side, formed by the jugal (*Os zygomaticum*). Two to three infraorbital foramina are visible ventral to the orbits and close to the maxillojugal suture (*sutura zygomaticomaxillaris*); they usually grow a bit more apart with increasing age. The premaxillomaxillar suture is kind of dome-shaped around the nasal opening, not extending as far dorsally as the nasals, leaving room for a short maxillanasal suture (*sutura nasomaxillaris*). The maxillafrontal suture (*sutura frontomaxillaris*) forms just dorsal to the nasals, often slightly zigzagging and extending ventrally into the orbits.

In lateral view in the juvenile skulls all the sutures between the visible bones are still unfused. The near 80°-orientation of the orbital plane (β) to the base of the skull is well visible in this view. The frontojugal suture (*sutura frontozygomatica*) is nearly straight at the orbital rim, but varies slightly in course in the postorbital constriction. In some skulls the frontal shows a sharply pointed triangular extension ventrally, just posterior to the orbital rim (MTD B19400), which is lacking in other skulls where the maxilla instead might show a small dorsal extension (Fig. 3c). Particularly variable

is the form of the dorsal extension of the sphenoid, which might be a narrow band between the maxilla and squamosum (e.g. EPRC 6-07) or a broadened wing-like extension with a dorso-posterior extension (e.g. MTD B24004). The jugal foramen (*foramen zygomaticofaciale*) is very close to this suture.

The zygomatic arch is slim and ends anterior to the external auditory meatus. The squamosal bone dorsal to the external auditory meatus is slightly bulged laterally but does not show a rim or stronger bulging visible in adult skulls. The parietosquamosal suture shows a marked ventrally pointing projection posterior to the external auditory meatus.

The distal view shows an inflated skull, the parieto-occipital suture and a marked bend within the occipital between posterior and ventral part of the skull. This bend develops to a strong lamdoidal crest in adults and ends laterally at the parietosquamosal suture.

In ventral view (Fig. 3d) all sutures between the bones are unfused. The large foramen magnum is posterior to the middle of the skull. In the juvenile skulls the pterygoid wings extend as far lateral as the maximal width of the snout. The most prominent of the visible foramina are the carotic canals in the bullae, circular and large, and the distal palatine foramina (*foramen palatinum majus*), situated in the maxillopalatine suture (*sutura palatinum transversa*). The glenoid fossa appears large and flat, distally terminated by a narrow pointed squamosal projection.

Mandible

The mandible of the juvenile skulls is characterized by the large alveoli for the still forming molars and the lateral tilt of the coronoid processes in dorsal view. The articular surface of the condyles is nearly horizontal, skewed oval, and no marked neck separates it from the mandibular ramus. The angular process is well rounded and does not extend as far posterior as the condyle. On the lingual side no pterygoid protuberances are visible. In lateral view the coronoid process is well pointed posteriorly and the bone between the tip of the coronoid process and the condyle is well rounded. A mental foramen (sometimes there are more) is visible in the lower part of the mandibular ramus ventral to dp3/4. The ventral rim of the mandible is nearly straight horizontally, slightly concave (Fig. 3e, f, g).

Description of subadult skull of *Pygathrix*

Cranium

The description is based primarily on skulls of a female of *P. cinerea* (EPRC 7-07) and a male of *P. nemaeus* (MTD B19854).

Most skull sutures are still well visible; in ventral view the maxillopremaxilla suture is about level to the dC/P1 junction; in the midline the palatinum extends forward to the M1/M2 junction; the large, oval palatine foramen are about level to the anterior part of M3.

In dorsal view the changes with increasing age are the following: the overall oval shape becomes more egg-shaped, that is more pointed anteriorly at the interorbital constriction, and more flattened posteriorly; the overall inflation of the skull extends further posterior and then bends ventrally more sharply.

The orbital rims increase in size and in dorsal view and form a flattened ledge in the anterior part of the skull: The postorbital constriction becomes more pronounced and a frontal crest forms. The snout is enlarged compared to the juvenile skull and also in relation to the cranium. This seems to be mainly due to an increased length of the maxilla leading to a lower inclination of the nose and

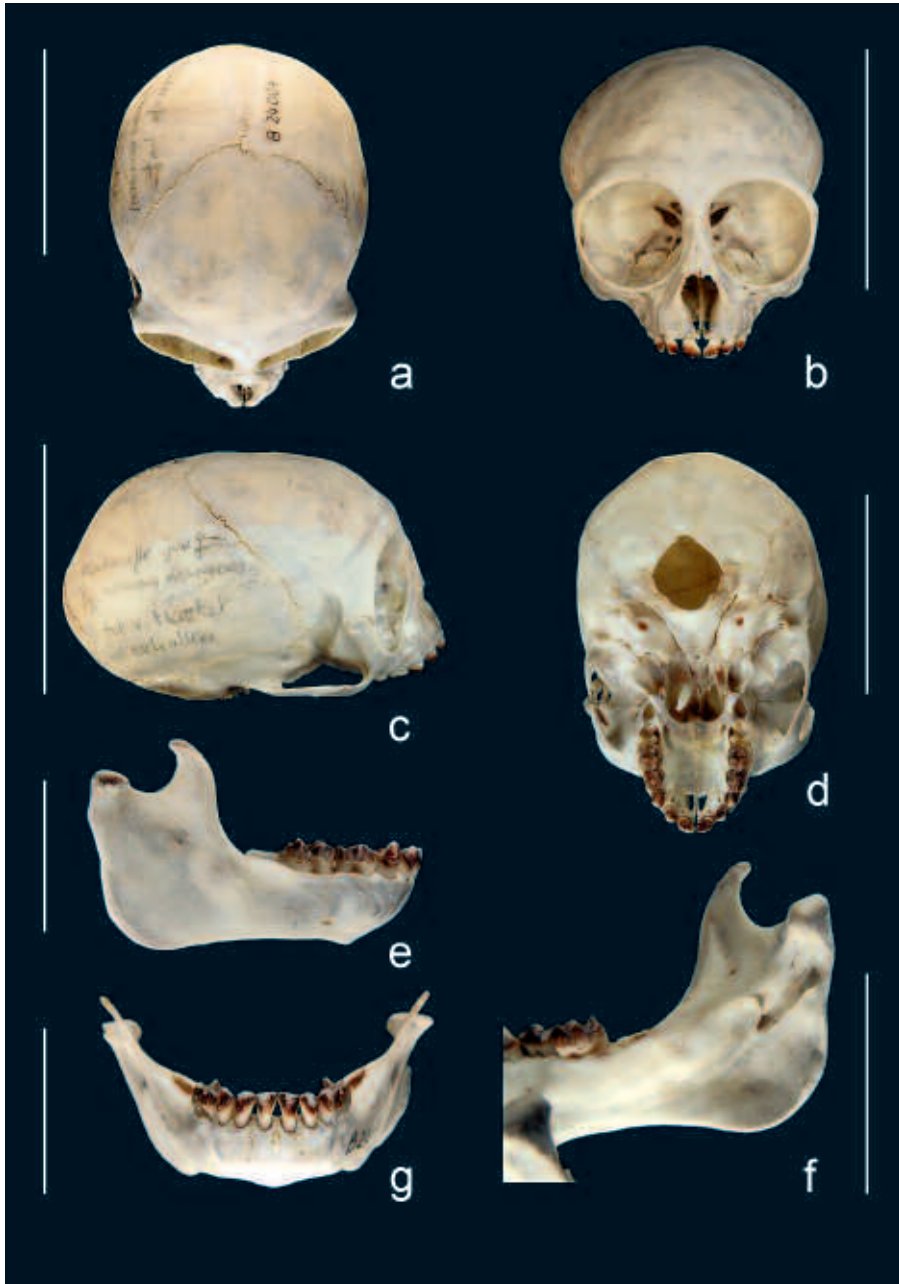


Fig.3. a. Skull of a juvenile female *Pygathrix nemaeus* (MTD B24004), dorsal view. Scale bar 5 cm; b. Dorso-frontal view; c. Lateral view; d. Ventral view; e. Mandible of a juvenile female *Pygathrix nemaeus* (MTD B24004), lateral view. Scale bar 2 cm; f. frontal view; g. lingual view. Photos: B. Bastian.

nasals (α about 70° in juveniles and about 55° in subadults), the same holds for the orbital plane (β 80° in juveniles and about 70° in subadults).

The orbits extend as far as, and with increasing age further lateral than the widest cranial width, thus dominate the frontal view. The zygomatic arch still ends anterior to the external auditory meatus but a tendency to form a crest or rim dorsal to the external auditory meatus is visible.

Mandible

The mandible is like in the juvenile skulls. But the pterygoid fossa on the medial side of the angular process is marked and structured in the very distal end with five slight grooves separated by well visible ridges (tuberositas pterygoidea); also at the angular process a crest develops at the ventral rim of the angular process. The coronoid process points distally, is slightly hook-shaped and shows a slight dorsal crest. There seems to be some variability in the form of the mandibular bone between the tip of the coronoid process and the condylar process: the distal pointing and thus strong appearance of a slim hook of the coronoid process of juvenile skulls is lost with increasing age and the coronoid process gets broader (with the broadening of the mandibular ramus as a whole), less pointed and ends blunt oriented dorsally.

Description of adult skull of *Pygathrix*

Cranium

The description is based primarily on skulls of a *P. nemaeus* male (EPRC 6-44) and a *P. nigripes* female (MTD B19857).

In dorsal view with increasing age frontoparietal crests form that run parallel towards the nuchal crest (Fig. 4a). They seem to be stronger in males, but this is difficult to judge as the exact age might also play a role and that is not known for the animals grouped as "adults" here. The orbital rims become more pronounced and extend further posteriorly in the form of a flattened ledge. This as well as the elongation of the rostral part result in the postorbital constriction being more pronounced and more or less in the middle of the skull length in the adult skulls compared to the juveniles, where this is in the anterior third of the skull. In the elongated rostral part of the skull the nasal opening is more oval and not so skewed oval as in the juvenile skulls.

In frontal view the orbitals are clearly broader than the greatest extension of the cranium and the greatest skull width in adult skulls is at the zygomatic arches (Fig. 4b).

In lateral view (Fig. 4c) the zygomatic arch extends with age more and more in a crest continuing dorsal to the external auditory meatus. The nuchal crest, not at the parieto-occipital suture but more ventral, extends laterally into a more or less marked crest extending nearly to the external auditory meatus. This lateral extension of the nuchal crest, however, does not fuse with the posterior extension of the zygomatic arch. Very variable is the course of the lacrimojugal suture in the orbita and the course of the maxillojugal suture.

In ventral view (Fig. 4d) the carotid canals (in the bullae) are oriented more centrally and open nearly straight ventrally and not so much towards the sagittal plane as in the juvenile skulls. The incisive foramen are more or less pointed anteriorly, more rounded to straight at the posterior end, and terminate posteriorly level to the posterior end of the canines. This relation to the canines is the same in juveniles.

Mandible

The mandible of the adult skull is more massive, but overall shows the same morphology as in subadults (Fig. 4e, f).

Age grouping and tooth eruption

The maxillary teeth present in juvenile and subadult skulls are listed in Table 3. The data suggest the sequence [M1 I1] [I2 M2] P4 P3 [M3 C] for the studied *Pygathrix* species. From the data it is

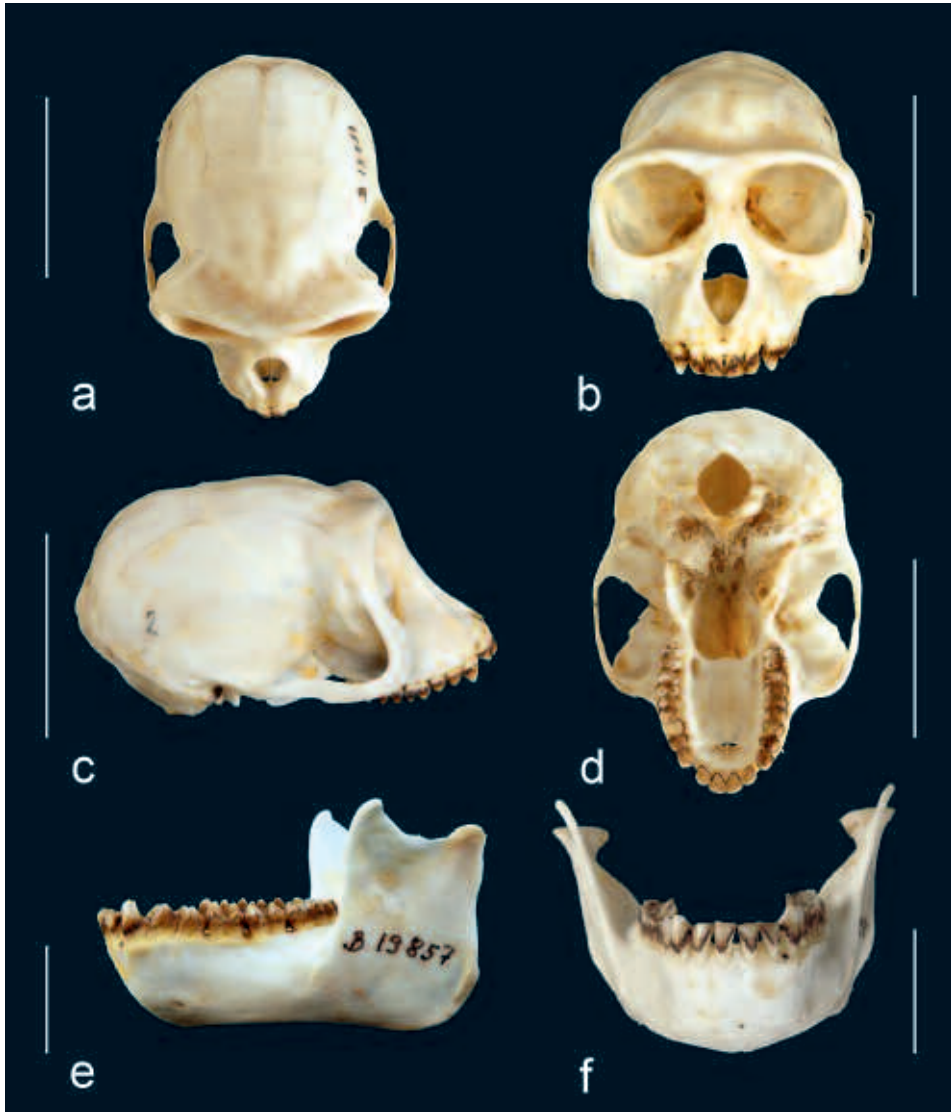


Fig.4. a. Skull of an adult female *Pygathrix nigripes* (MTD B19857), dorsal view. Scale bar 5 cm; b. frontal view; c. lateral view; d. ventral view; e. Mandible of an adult female *Pygathrix nigripes* (MTD B19857), lateral view. Scale bar 2 cm; f. frontal view. Photos: B. Bastian.

very difficult to clearly determine the sequence of M1 and I1 as in some very young individuals the presence of M1 was not verifiable. It is equally difficult to clearly determine the sequence of I2 and M2 as both are emerging in the relevant specimen (EPRC 6-X3). P4 seems to erupt prior to P3 in EPRC 6-24 and 7-07. Similarly the sequence of C and M3 is difficult to determine, in one specimen (EPRC 7-26 both are emerging and in one (EPRC 7-20) M3 is erupted and C still emerging. As the first is a female and the second a male there might be sexual differences involved. In *Pygathrix nemaeus* one specimen lacks only the canine of the final dentition, whereas one specimen of *P. cinerea* at the age of 8 years still shows dC, DP3 and an emerging P4.

Table 3. Teeth present in the maxillar dentition of some studied individuals. (Age* - indicates fairly exact age known. Teeth: d –deciduous tooth; em – emerging, not quite in occlusion yet; in alv – visible in alveole; x – present; ? status not known; n – not visible).

EPRC number	Sex	Age group	Age	I1	I2	C	P3	P4	M1	M2	M3
<i>Pygathrix cinerea</i>											
7-12	m	juv	1 year*	dl	dl	dC	dP	dP	in alv	n	n
7-20	m	subad	5 years	dl	dl	em	x	x	x	x	x
7-18	m	subad	5 years	x	x	x	x	x	x	x	x
7-33	m	ad	8 years	x	x	x	x	x	x	x	x
7-02	m	ad	11 years	x	x	x	x	x	x	x	x
7-07	f	subad	?	x	x	dC	dP	em	x	x	n
7-26	f	ad	?	x	x	em	x	x	x	x	em
<i>Pygathrix nemaeus</i>											
6-22	m	newborn	1 day*	dl	dl	dC	dP em	dP	in alv	n	n
6-11	m	newborn	5 days*	dl	dl	dC	dP	dP	?	n	n
6-43	m	juv	8 months*	dl	dl	dC	dP	dP	?	n	n
6-X3	m	juv	?	x	em	dC	dP	dP	x	em	n
6-19	m	juv	2 years	dl	dl	dC	dP	dP	x	em	n
6-27	m	juv	3 years*	l	dl?	dC	dP	dP	x	em	n
6-25	m	juv	3,5 years*	x	x	dC	dP	dP	x	x	em
6-08	m	subad	5..6 years	x	x	dC	x	x	x	x	x
6-44	m	ad	?	x	x	x	x	x	x	x	x
6-20	f	juv	1 year	dl	dl	dC	dP	dP	in alv	n	n
6-24	f	juv	3,5 years*	x	x	dC	dP	in alv	x	x	n
6-18	f	juv	3 years	dl	dl	dC	dP	dP	?	?	?
6-17	f	ad	10 years	x	x	x	x	x	x	x	x
6-40	f	ad	?	x	x	x	x	x	x	x	x
<i>Pygathrix nigripes</i>											
13-04	f	juv	2 years	dl	dl	dC	dP	dP	in alv	n	n
13-06	f	juv	2 years	dl	dl	dC	dP	dP	in alv	n	n
MTD B19857	f	ad	?	x	x	x	x	x	x	x	x

Craniometry and Statistics

The cranial morphology of the three species of douc langurs is very similar and so are the measurements (Fig. 5). In overall skull geometrics as illustrated by a scatter diagram of zygomatic width against greatest skull length, no difference between the species can be observed (Fig. 6). Most of the original measurements show a normal or near normal distribution for the studied species (except of *P. nigripes* where the sample was considered too small for these tests). Only cbf and nb showed no normal distribution in *P. cinerea* and were not used in further analyses.

A sexual dimorphism can be seen in *P. cinerea* and *P. nemaeus* but seems to be markedly stronger in *P. cinerea*. In this species 17 original measurements differed significantly between the sexes in age class 2 and 3, 19 age- independent residuals and 2 log transformed variables.

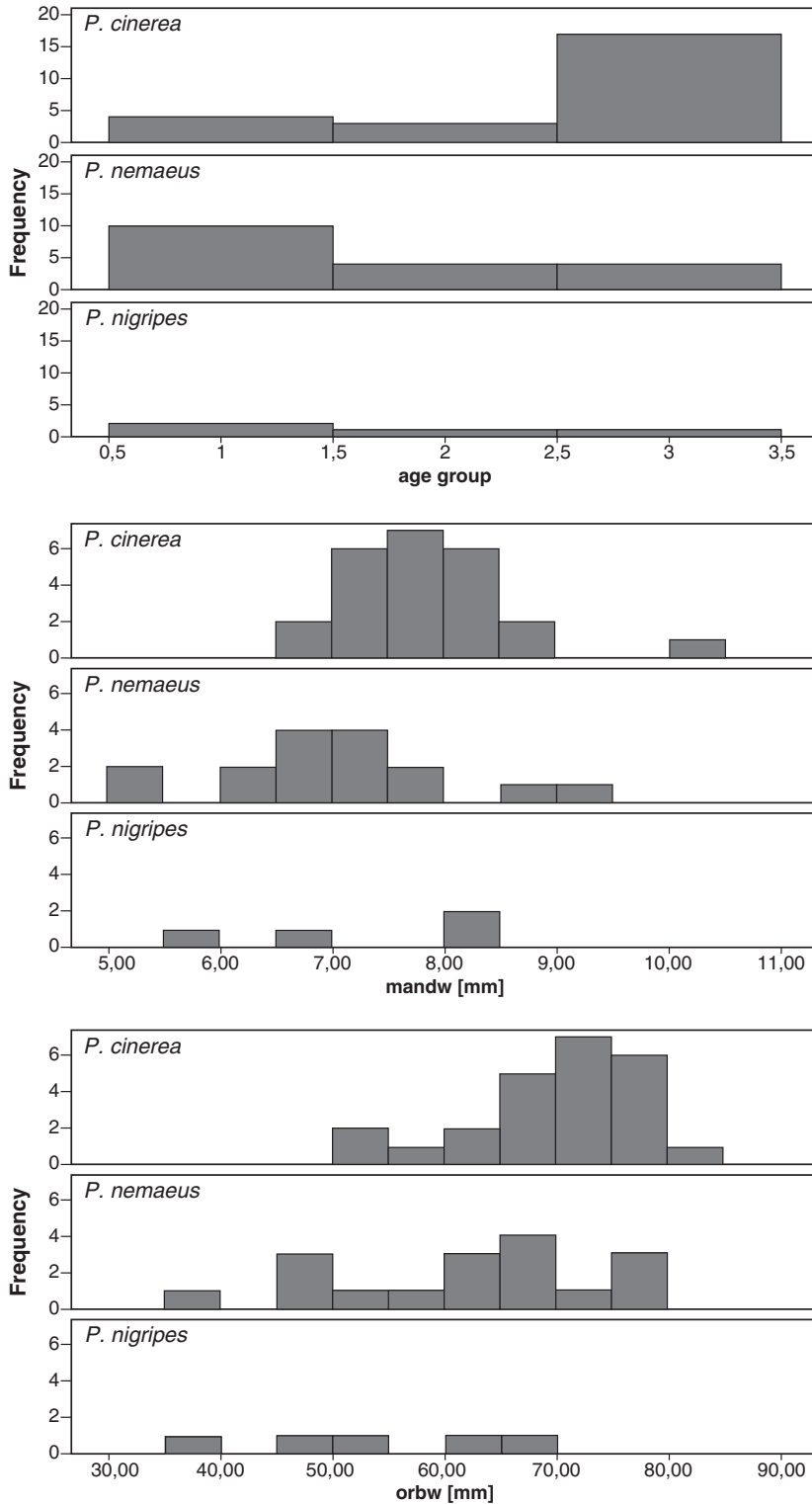


Fig.5. Frequency of age groups, orbital width (orbw) and mandibular width (mandw).

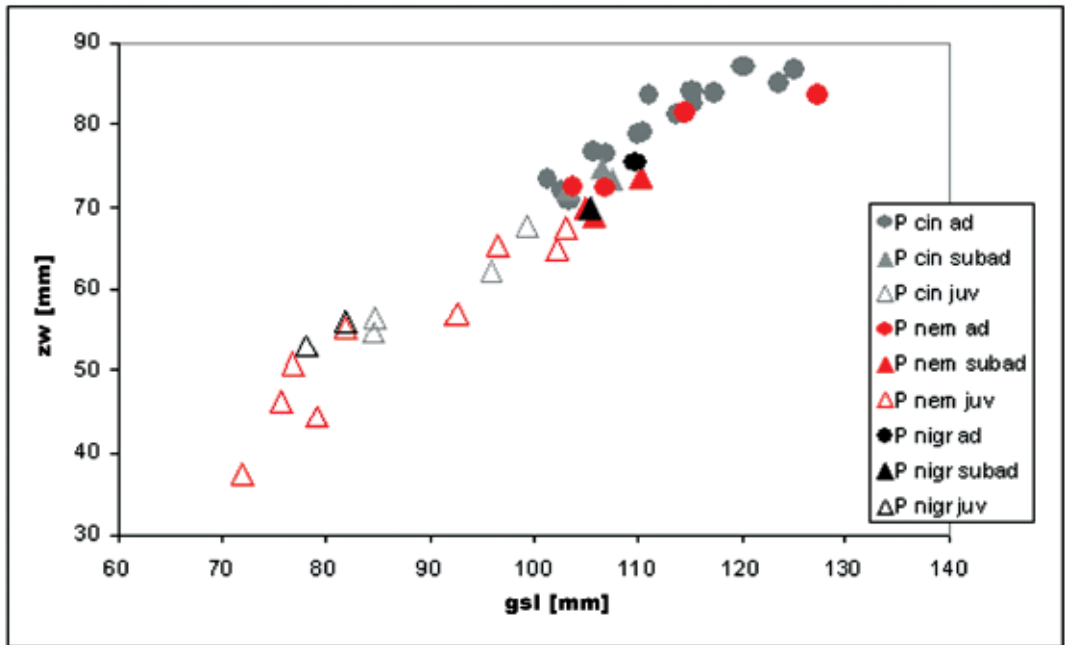


Fig.6. Scatter diagramm of zygomatic width against greatest skull length for *Pygathrix cinerea* (P cin), *P. nemaeus* (P nem) and *P. nigripes* (P nigr) separated for age groups: ad – adult, subad – subadult, juv – juvenile.

In *Pygathrix nemaeus* the sexual dimorphism seems to be weaker, only in one age-independent residual (RES-cm3) a statistically significant difference between males and females was observed.

The PCAs resulted in several factors, the first four explaining > 90% of the total variance for the original data in age classes 2 and 3 as well as for the age-independent residuals and log-transformed data. In all cases maxb, zw, pcp, gsl, orb, libasio, lifm, cbf and lip contributed most to the first factor. Also important were dm3, and pmaxb.

The DAs show some degree of separation of the three studied species of *Pygathrix*. Using the variables mainly associated with PCA factor 1 and specimens of age class 2 and 3, the species show a clear separation with the original data but *P. nigripes* is not well represented. (Fig. 7A). The distinction of *P. nemaeus* and *P. cinerea* is weaker and *P. nigripes* again not well represented with the age-independent residuals important in the first factor of the PCA (Fig. 7B). Using the log transformed data which explain most of the variance in PCA factor 1 does not give a separation of the species. Using some random age-independent residuals in a DA indicates a slightly better separation of the three species without ungrouped cases (Fig. 7C). But still there is some overlap between *P. cinerea* and *P. nemaeus* and *P. nigripes* well separated from the others. Reducing the number of residuals used in the DA did not increase the possibility to separate between the species. Using a number of random log transformed variables a separation of *P. nigripes* from the others but no clear separation of *P. cinerea* and *P. nemaeus* is possible (not shown).

Discussion

So far only Harding (2003) provided some craniometric data of *Pygathrix*. Her sample consisted of 12 *P. nemaeus*, 10 *P. nigripes* and 4 *P. cinerea*, thus the number of specimens of the species differed from this study. Here we give a more detailed description of cranial morphology and

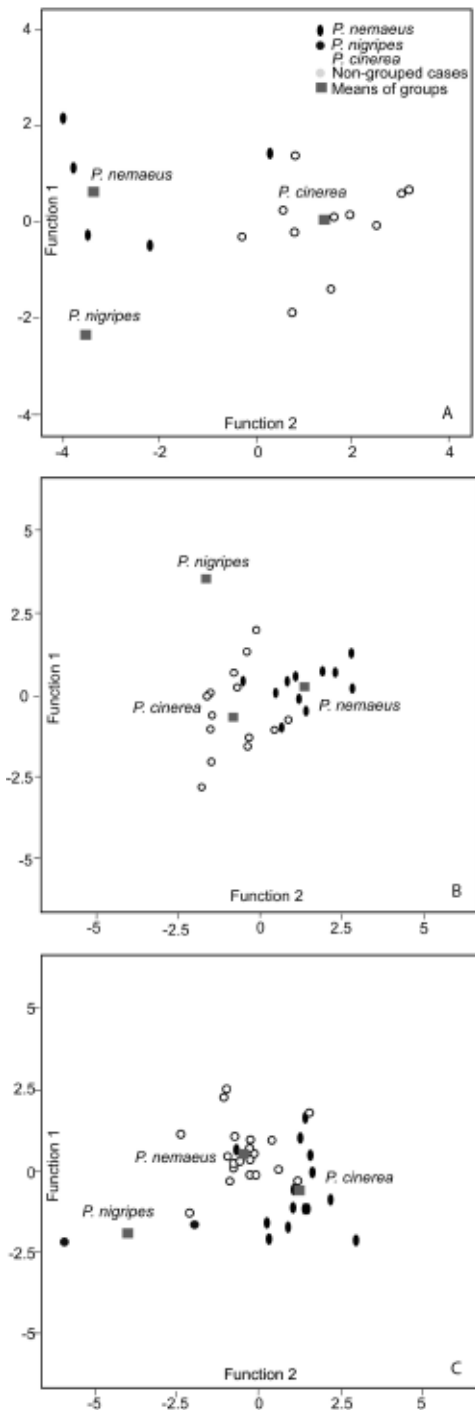


Fig.7. Discriminant analyses of the three species of *Pygathrix* with different sets of data. **A:** with original measurements as given in the structure matrix and specimens of age class 2 and 3 only; **B:** with age-independent residuals of some variables as given in the structure matrix and all specimens; **C:** with a set of random age-independent residuals of some variables as given in the structure matrix and all specimens.

ontogenetic changes as well as more data on skull morphometrics. The three species of *Pygathrix*, can be separated on the basis of coat color, few morphological features and molecular genetics (Nadler, 1997; Roos & Nadler, 2001; Harding, 2003; Roos, 2004). The cranial morphology of the three species, however, is very similar, so that in gross or overall morphology no diagnostic features were found to allow for a species differentiation. For statistical analysis the studied samples are not ideal as the specimens are of different and often unknown ages and the three species are not represented in sufficient and similar numbers which could not be helped during the course of the study. Results can nevertheless be compared to Harding (2003) who studied samples of similar unequal sizes. The linear measurements of cranial parameters are hardly distinguishable but boxplots of some measurements indicate that *P. cinerea* is the largest and *P. nigripes* a smaller species with *P. nemaeus* having the largest range (Fig. 6). This is in accordance to Harding (2003).

The principal component analyses with the original data, age-independent residuals and log-transformed variables pointed to the main importance of the same variables to explain most of the overall variability in the sample. These include length and width measurements of the skull and the height and width of the mandibular ramus thus variables influencing overall geometry.

Discriminant analyses using the variables which mainly influenced the PCA factor 1 gave different results in the DAs. The log transformed data did not reveal a clear separation of the species at all. Using original data and specimens of the age classes 2 and 3, thus adult specimens only or age independent residuals indicated a separation of the three species to a different degree. In all cases (Fig. 7) *P. nigripes* was further separated than the other two species. Due to the small sample size studied here this has to be treated with great care but is in accordance to Harding (2003) who stated that "*P. nigripes* appears to be the most distinctive species" on the base of external differences though.

One aspect that might influence the

differentiating power of the DAs with the selected variables is the difference in sexual dimorphism in the samples of *P. cinerea* and *P. nemeaus*.

The DA's indicate that there might be the possibility for a separation of the three species on the base of cranial measurements but only with larger and similar sized samples of all species and possibly also including methodology of geometric morphometrics which might be more suitable to pick up differentiating signals between the species. That was not feasible in the study presented here but might be future approach.

The statistical tests yielded a strong sexual dimorphism in *P. cinerea* but a weak one in *P. nemeaus*. As both species are closely related these results are surprising and hard to explain. As the sample of *P. nemeaus* contained more juvenile specimens sexual dimorphism might be more pronounced in older specimens than were available. Pan & Oxnard (2001) indicated some sexual dimorphism for *P. nemeaus* but their measurements are difficult to compare to those used in the present study. These authors also stated (for *P. nemeaus* and several species of *Rhinopithecus*) that sexual dimorphism is often related to width variables, species separation rather to length variables. This cannot be verified in the present study as there were several sexual differences in *P. nemeaus* and hardly any between species. In the DAs the functions separating the species are influenced by length and width variables likewise.

A problem of the presented study is the material of different ages which had been addressed in different ways. Using age groups 2 and 3 only had the disadvantage of having even less specimens but an at least more even size range, as "adult" size is reached at about 4 years. The DAs indicated differentiation of the species of these age groups. The other approach was to use age-independent residuals of a linear regression to age groups. This approach had the problem that the age classes are not all equal. The residuals however showed some power of differentiation of the species. The last approach was the use of log transformed data which did not show a differentiation of species in the DAs. It seems that larger samples of adult specimens would probably be best for a future study on species differentiation.

Overall the results of the presented study show that the skulls of the three species of *Pygathrix* are hardly distinguishable on the basis of morphology alone, but that cranial measurements reveal some potential to separate the species in DAs.

The dental eruption sequence of permanent maxillar teeth has been given as M1 [I1 M2] I2 P3? P4 C M3 by Harvati et al. (2000) and Swindler (2002). Harvati et al. (2000) indicate that the eruption sequence was not determinable at the P3/P4 position. In one individual studied here it is indicated that P4 emerges prior to P3. Another specimen indicates that M3 emerges prior to C. Harvati et al. (2000) state that *Trachypithecus cristatus* completes dental development at the age of about 3.75 years and *Macaca fascicularis* at about 5.5 years, thus later. With the specimens studied here it is still difficult to determine the age when dental development ends in *Pygathrix*: one specimen of *P. cinerea* shows complete adult dentition at the age of about 5 years, a specimen of *P. nemeaus* still has a dC at the age of about 6 years, a *P. cinerea* of 5 years shows dl1, dl2 and dC. An even larger sample of immature specimens that demonstrate various stages of dental eruption is still needed to further clarify the normal eruption sequence, or to better understand the variability that is indicated.

Acknowledgements

We thank Mr. Truong Quang Bich, Director of Cuc Phuong National Park and Mr. Do Van Lap, Chief of the Department Scientific and International Collaborations for arrangements to work at the

Endangered Primate Rescue Center and Mr. Vu Ngoc Thanh, Director of the Zoological Museum at Hanoi National University for permission and support to involve the material of the Zoological Museum in our study.

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